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PRODUCT DATASHEET

Ready-to-Assay™ Mu Opioid Receptor Frozen Cells

CATALOG NUMBER: HTS101RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

Opiates derived from the opium poppy, *Papaver somniferum*, have been used in for millenia for their anti-diarrheal, analgesic and euphoric properties. More recently, endogenous peptides, enkephalins, dynorphins, and endorphins, were found to bind to the same sites as opiate alkaloids. The receptors for the classical opioids are three related GPCRs, μ, κ, and δ, that activate Gi/o to reduce intracellular cAMP levels. Most clinically used opioids function by activation of the μ opioid receptor (Dhawan *et al.*, 1996). Cloned human μ-expressing cell line is made in the Chem-5 host, which supports high levels of recombinant μ expression on the cell surface and contains optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at μ.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

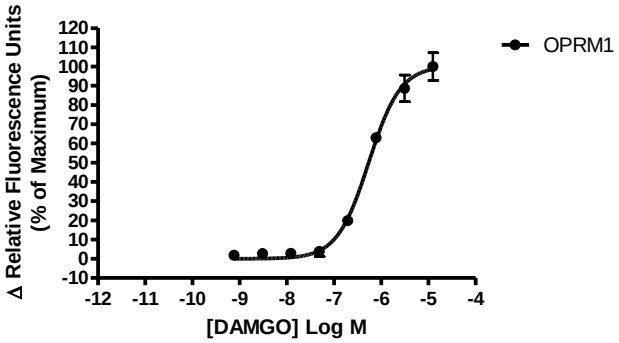

Figure 1. Representative data for activation of μ-opioid receptor. Calcium flux in μ opioid–expressing Chem-5 cell line induced by DAMGO. μ opioid–expressing Chem-5 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,000 RLU (Relative Light Units).

Table 1. EC_{50} value of μ-opioid -expressing Chem-5 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
DAMGO	Calcium Flux	550	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
DAMGO ligand	Sigma: D8040
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for $FLIPR^{TETRA}$® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-5, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein as well as an exogenous proprietary promiscuous Gα protein.

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EXONGENOUS GENE EXPRESSION

OPRM1 cDNA (Accession Number: NM_000914; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                  ATG TCA GAT GCT CAG CTC GGT CCC CTC CGC
                                                   M   S   D   A   Q   L   G   P   L   R
ACG CTC CTC TCT GTC TCA GCC AGG ACT GGT TTC TGT AAG AAA CAG CAG GAG CTG TGG CAG CGG CGA
 T   L   L   S   V   S   A   R   T   G   F   C   K   K   Q   Q   E   L   W   Q   R   R
GAA GCG GCT GAG GCG CTT GGA ACC CGA AAA GTC TCG GTG CTC CTG GCT ACC TCG CAC AGC GGT GCC
 E   A   A   E   A   L   G   T   R   K   V   S   V   L   L   A   T   S   H   S   G   A
CCG GCC GTC AGT ACC ATG GAC AGC AGC GCT GCC CCC ACG AAC GCC AGC AAT TGC ACT GAT GCC TTG
 P   A   V   S   T   M   D   S   S   A   A   P   T   N   A   S   N   C   T   D   A   L
TAC TCA AGT TGC TCC CCA GCA CCC AGC CCC GGT TCC TGG GTC AAC TTG TCC CAC TTA GAT GGC AAC
 Y   S   S   C   S   P   A   P   S   P   G   S   W   V   N   L   S   H   L   D   G   N
TCC GAC CCA TGC GGT CCG AAC CGC ACC GAC CTG GGC GGG AGA GAC AGC CTG TGC CCT CCG ACC GGC
 S   D   P   C   G   P   N   R   T   D   L   G   G   R   D   S   L   C   P   P   T   G
CCC TCC ATG ATC ACG GCC ATC ACG ATC ATG GCC CTC TAC TCC ATC GTG TGC GTG GTG GGG CTC TTC
 P   S   M   I   T   A   I   T   I   M   A   L   Y   S   I   V   C   V   V   G   L   F
AAC TTC CTG GTC ATG TAT GTG ATT GTC AGA TAC ACC AAG ATG AAG ACT GCC ACC AAC ATC TAC ATT
 N   F   L   V   M   Y   V   I   V   R   Y   T   K   M   K   T   A   T   N   I   Y   I
AAC CTT GCT CTG GCA GAT GCC TTA GCC ACC AGT ACC CTG CCC TTC CAG AGT GTG AAT TAC CTA ATG
 N   L   A   L   A   D   A   L   A   T   S   T   L   P   F   Q   S   V   N   Y   L   M
ACA TGG CCA TTT GGA ACC ATC CTT TGC AAG ATA GTG ATC TCC ATA GAT TAC TAT AAC ATG TTC ACC
 T   W   P   F   G   T   I   L   C   K   I   V   I   S   I   D   Y   Y   N   M   F   T
ATA TTC ACC CTC TGC ACC ATG AGT GTT GAT CGA TAC ATT GCA GTC TGC CAC CCT GTC AAG GCC TTA
 I   F   T   L   C   T   M   S   V   D   R   Y   I   A   V   C   H   P   V   K   A   L
TTC CGT ACT CCC CGA AAT GCC AAA ATT ATC AAT GTC TGC AAC TGG ATC CTC TCT TCA GCC ATT GGT
 F   R   T   P   R   N   A   K   I   I   N   V   C   N   W   I   L   S   S   A   I   G
CCT GTA ATG TTC ATG GCT ACA ACA AAA TAC AGG CAA GGT TCC ATA GAT TGT ACA CTA ACA TTC TCT
 P   V   M   F   M   A   T   T   K   Y   R   Q   G   S   I   D   C   T   L   T   F   S
CCA ACC TGG TAC TGG GAA AAC CTG CTG AAG ATC TGT GTT TTC ATC TTC GCC TTC ATT ATG CCA GTG
 P   T   W   Y   W   E   N   L   L   K   I   C   V   F   I   F   A   F   I   M   P   V
ATC ATT ACC GTG TGC TAT GGA CTG ATG ATC TTG CGC CTC AAG AGT GTC CGC ATG CTC TCT GGC TCC
 I   I   T   V   C   Y   G   L   M   I   L   R   L   K   S   V   R   M   L   S   G   S
GAA AAG GAC AGG AAT CTT CGA AGG ATC ACC AGG ATG GTG CTG GTG GTG GTG GCT GTG TTC ATC GTC
 E   K   D   R   N   L   R   R   I   T   R   M   V   L   V   V   V   A   V   F   I   V
TGG ACT CCC ATT CAC ATT TAC GTC ATC ATT AAA GCC TTG GTT ACA ATC CCA GAA ACT ACG TTC CAG
 W   T   P   I   H   I   Y   V   I   I   K   A   L   V   T   I   P   E   T   T   F   Q
GTT TCT TGG CAC TTC TGC ATT GCT CTA GGT TAC ACA AAC AGC TGC CTC AAC CCA GTC CTT TAT GCA
 V   S   W   H   F   C   I   A   L   G   Y   T   N   S   C   L   N   P   V   L   Y   A
CTG GAT GAA AAC TTC AAA CGA TGC TTC AGA GAG TTC TGT ATC CCA ACC TCT TCC AAC ATT GAG CAA
 L   D   E   N   F   K   R   C   F   R   E   F   C   I   P   T   S   S   N   I   E   Q
AAC TCC ACT CGA ATT CGT CAG AAC ACT AGA GAC CAC CCC TCC ACG GCC AAT ACA GTG GAT AGA ACT
 N   S   T   R   I   R   Q   N   T   R   D   H   P   S   T   A   N   T   V   D   R   T
CAT CAG CTA GAA AAT CTG GAA GCA GAA ACT GCT CCG TTG CCC TGA
 H   Q   L   E   N   L   E   A   E   T   A   P   L   P  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS101M	ChemiScreen™ Mu Opioid receptor membrane prep

* Note: Chem-5 cells are derived from Chem-1 cells

REFERENCES

1. Dhawan BN *et al.* (1996) International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* 48: 567-92.

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